Quantitative Evaluation of Microalgal Cell Disruption for Hydrocarbon Extraction

	つつみ　しゅん
氏　　名	堤　駿
授　与　学　位	博士（工学）
学位授与年月日	平成３０年３月２７日
学位授与の根拠法規	学位規則第４条第１項
研究科，専攻の名称	東北大学大学院工学研究科（博士課程）化学工学専攻
学位論文題目	Quantitative Evaluation of Microalgal Cell Disruption for Hydrocarbon Extraction (炭化水素抽出のための微細藻類の細胞破砕の定量的評価)
指　導　教　員	東北大学教授　青木　秀之
論文審査委員	主査　東北大学教授　青木　秀之　東北大学教授　猪股　宏 東北大学教授　塚田　隆夫

論 文 内 容 要 旨

【Chapter 1】The colonial microalga, *Botryococcus braunii* produces and stores long-chain hydrocarbons (triterpenoids) in membranes and colonies. Thus, it is expected to be used as a biofuel feedstock. However, since algal cell suspension is dilute, a large amount of energy is required to extract hydrocarbons which are converted to biofuels [1]. To extract them for low input energy, developing pretreatment process for wet biomass prior to the extraction (e.g., cell disruption) is crucial. Numerous pretreatment methods were suggested in previous studies. However, because the effectiveness of the pretreatment is significantly influenced by several factors such as algal species and an organic solvent, it is difficult to determine the best pretreatment methods for microalgal lipid extraction. Thus, the accumulation of knowledge regarding several pretreatment methods performed under the several algal conditions is necessary for future process design. In this thesis, mechanical cell disruption to *B. braunii* was investigated considering scalability and cost-effectiveness. First of all, algal cells were disrupted using three disruption devices, and the effectiveness of cell disruption on hydrocarbon extraction was evaluated (Chapter 2). Then, factors influencing on the hydrocarbon extraction were investigated, and the prediction of hydrocarbon yield was considered (Chapter 3). Individual cell strength of *B. braunii* was measured, and that was compared to the other microalgae (Chapter 4).

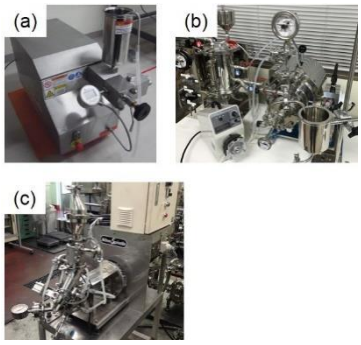

Fig. 1 Cell disruption devices.
(a) High pressure homogenizer,
(b) Bead mill, (c) JET PASTER

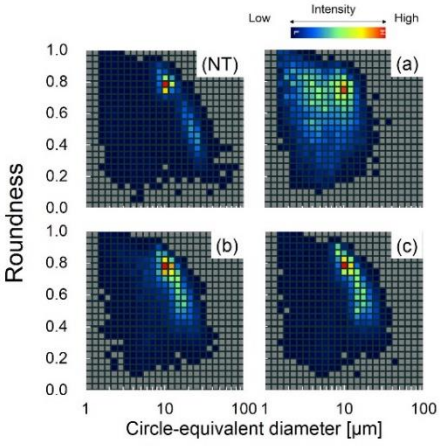

Fig. 2 Particle properties. (NT) Untreated, (a) High pressure homogenizer, (b) Bead mill, (c) JET PASTER

【Chapter 2】To investigate the effectiveness of the disruption of *B. braunii* cells, three cell disruption devices (Fig. 1) were used, and hydrocarbons in the

cells were extracted. The high-pressure homogenizer induces shear force, impulsive forces caused by impingement of fluid and cavitation. The bead mill disrupts materials in liquid by shear forces between rigid beads and algal cells. The JET PASTER disperses particles by intensive mixing. The JET PASTER applies shear force and impulsive force caused by cavitation. The particle size and shape of samples before and after the cell disruption were measured, and the influence of cell disruption on algal morphology was investigated. The morphological change in particles after each treatment is shown in Fig. 2. The particle diameter of *B. braunii* became small after the treatments irrespective of the device used. Considering the diameter of *B. braunii* cell was approximately 10 μm, algal colonies were disrupted. Although cells were significantly disrupted by the homogenizer (55% of the degree of cell disruption), cells were slightly or not disrupted in the bead mill or JET PASTER treatment. In contrast, the hydrocarbon yield of disrupted sample (Fig. 3) drastically increased compared to the untreated one in all the conditions (e.g., 2.7% to 82.8% after the JET PASTER treatment). Thus, irrespective to whether the cells were disrupted, the colony disruption increased the hydrocarbon yield. In the previous study [2], the extracellular polysaccharides (EPS) produced by *B. braunii* is known as preventing the hydrocarbon extraction. Thus, focusing on not only colony disruption but the algal surface structure, the microscopic observation of negative staining using India ink was performed. Although the EPS existed tightly around its colony in the untreated sample, the EPS were removed in the disrupted sample. This caused low hydrocarbon yield from the untreated sample and increased the yield after the disruption. Above facts suggested that the disruption of algal colonies was effective for the extraction of a large amount of hydrocarbons while the fractionation of cells is not needed for hydrocarbon extraction from *B. braunii*.

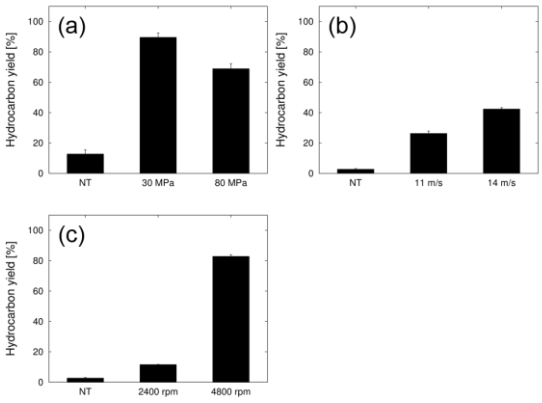

Fig. 3 Hydrocarbon yield from cell culture. (a) High pressure homogenizer, (b) Bead mill, (c) JET PASTER

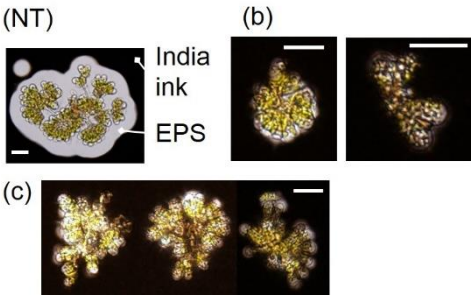

Fig. 4 Negative staining images. Size bar: 20 μm (NT) untreated, (b) bead mill, (c) JET PASTER

【Chapter 3】 Developing the useful pretreatment method effectively requires the prediction method of hydrocarbon yield which does not depend on the complicated hydrocarbon extraction and the quantification and observation of EPS. The algal EPS contains acidic sugars (uronic acids) which have carboxyl functional group [3]. Thus, the EPS is ionized and charged negatively depending on the culture pH. Although cellulose which is the main component of cell wall does not have the carboxyl group, the cell wall is expected not to be ionized significantly compared to the EPS. Based on this nature, the removal of EPS was evaluated by zeta potential. In this chapter, particle size distribution and zeta potential were used for quantifying the degree of colony disruption and the change in surface structure, simultaneously. Indicating that the colony disruption and EPS removal, enhanced hydrocarbon yield from wet *B. braunii* in Chapter 2,

the relationships of the colony disruption and surface structure to the hydrocarbon yield were investigated. The zeta potential of algal samples without pretreatment was -30.5 mV at pH 9, while that with pretreatment ranged from -29.7 to -18.8 mV. While the algal zeta potential to the culture pH is lower depending on the increase of pH, the change in the degree of zeta potential was different according to the pretreatment methods. This is because that the removal degree of the EPS was different from the pretreatments. Moreover, when the relationship between the zeta potential and the amount of the removed EPS quantified by a phenol-sulfuric acid method was investigated, the linear relationship was observed between them (R-squared value = 0.96). And then, the relationship between the zeta potential and hydrocarbon yield was investigated, the higher zeta potential indicated higher hydrocarbon yield. However, in the sample treated by the JET PASTER, hydrocarbon yield increased largely despite its low zeta potential. To investigate the colony disruption quantitatively, particle size distributions were used as indicators of the degree of colony disruption. When number-size distribution and volume-size distribution of the same sample were compared (Fig. 5), the median diameters were significantly different. This is because that the number-size distribution emphasizes small particles and the volume-size one emphasizes large particles. For *B. braunii* culture, the existence of algal cells and colonies which were formed by the aggregation of single cells caused the difference in the distributions. Here, the relationship between the change in median diameter and hydrocarbon yield except for the thermal heating where particle size did not change at all was investigated (Fig. 6), observing the exponential relationship (R-squared value = 0.81). Above facts suggested that: (i) using algal zeta potential for evaluating the removal of EPS was effective, (ii) using algal zeta potential for the prediction of hydrocarbon yield was appropriated for the pretreatments that did not influence the particle size such as thermal heating, and (iii) using particle size distribution for the prediction was suitable for the pretreatments that changed particle size such as cell disruption.

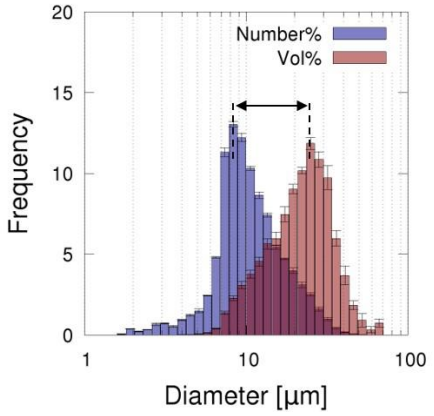

Fig. 5 Comparison of Particle size distributions.

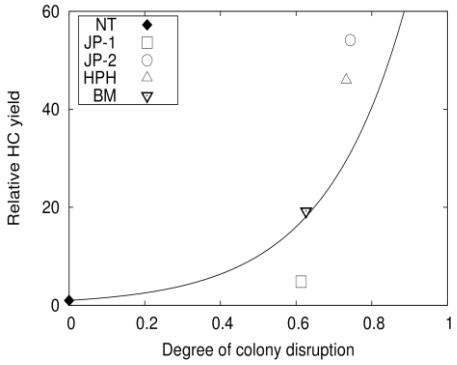

Fig. 6 The relationship between particle property and hydrocarbon yield.

【Chapter 4】 Understanding cell mechanical strength can decrease the excess input energy. In this chapter, nanoindentation method for measuring mechanical cell strength was performed. The nanoindentation device compresses materials vertically and detects the displacement of the prove according to the applied force. In this experiment, the cell fixed on the acryl chamber was compressed vertically in the culture medium, and the force-displacement curves until 5 mN was applied to the cells were obtained. When the cell burst, the specific point was observed shown in Fig. 7. The force at this point was defined as cell strength, and the mechanical strengths of approximately 30 number of cells

were measured. The strength and conventional stress obtained via the experiment were summarized in Table 1. These values were 15–33 times higher than those of the other microalgae reported in the previous study [4], which was derived from the cell structure of *B. braunii*. *B. braunii* has two types of cell walls that have different thickness (one of the cell wall thickness was 50–55 and the other was 1000 Å [5]), and the cell walls formed double layer structure [6, 7]. Hence, the strength of *B. braunii* cell would be reinforced. In contrast, when the energy for disrupting 1 g of cells was calculated from the strength and displacement, the value of *B. braunii* was lower or similar to that of other algae reported in the previous study. This is because that the cell size of *B. braunii* (8–10 μm) were larger than those of the other microorganisms reported in the Overbeck's study (3–5 μm), resulting in small number of cells in the same dry cell weight. When the cell disruption degree using a high-pressure homogenizer, one of practical cell disruption devices, was evaluated by measuring light absorbance of metabolite released to cell culture, the cell disruption degree increased with the treatment pressure at above 30 MPa, and 70% of cells were disrupted at 80 MPa. These values were small compared to the other microorganisms (146 MPa of homogenized pressure was required for 50% of the disruption of *S. cerevisiae* cells) [8, 9], which were the same species reported by Overbeck's study. This inconsistency to the facts of the cell strength would be derived from the algal cell size. The disruption principle of the high-pressure homogenizer is that particles are forced into the narrow valve gap the size of which was approximately 8 μm at 80 MPa [10], being smaller than cell size of *B. braunii*. On the other hand, the cell size of *S. cerevisiae* was approximately 5 μm, which is smaller than the valve gap. Those facts indicate that the cell size was important for the cell disruption using the high-pressure homogenizer.

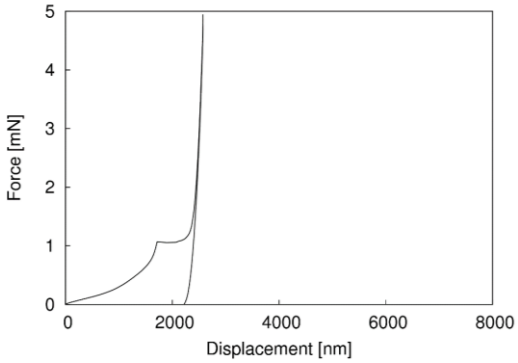

Fig. 7 Typical force-displacement curve.

Table 1 Single-cell compression data.

d_C	[mm]	8.93 ± 0.842
F_B	[mN]	3574 ± 921
P_B	[MPa]	57.6 ± 16.2
W_{spec}	[J/g]	3.25

All values represent mean ± SD.

【Chapter 5】 In this thesis, the cell disruption of *B. braunii* was performed, and the effectiveness and fundamental algal parameters for developing useful method were investigated. The removal of extracellular polysaccharides from colony and disruption of colony using three disruption devices enhanced hydrocarbon yields. Measuring the cell mechanical strength enabled to estimate the energy for disrupting 1 g of algal cells.

【Nomenclature】 d_c: cell size, F_B: the force at cell burst, P_B: the conventional stress, W_{spec}: the energy for disrupting 1 g of cells

【References】 [1] Lardon et al., *Environ. Sci. Technol.*, **43**, 6475 - 6481 (2009) [2] Furuhashi et al., *Algal Res.*, **16**, 160-166 (2016), [3] Atobe et al., *J. Appl. Phycol.*, **27**, 755-761 (2014), [4] Overbeck et al., *Chem. Eng. Technol.*, **40**, 1158-1164 (2017), [5] C. Berkaloff et al., *Phytochemistry*, **22**, 389-397 (1983), [6] C. Largeau et al., *Phytochemistry*, **19**, 1043-1051 (1980), [7] T. Tanoi et al., *J. Appl. Phycol.*, **26**, 1-8 (2013), [8] E. M. Spiden et al., *Bioresour. Technol.*, **140**, 165-171 (2013), [9] E. M. Spiden et al., *Biochem. Eng. J.*, **70**, 120-126 (2013), [10] A. R. Kleinig and A. P. J. Middelberg, *Chem. Eng. Sci.*, **51**, 5103-5110 (1996)

論文審査結果の要旨

微細藻類 *Botryococcus braunii* の炭化水素は燃料の原料として注目されているものの、微細藻類の培養液は希薄であり、バイオ燃料の原料となる炭化水素の抽出には多大なエネルギーを要する。低消費エネルギーで多量の炭化水素を抽出するには、培養液中での藻類の破砕をはじめとした抽出前処理プロセスの開発が重要である。このような前処理方法には、多くの研究例が検討されているものの、有効な方法は藻類や抽出溶媒の種類などに大きく影響されるため、様々な方法を藻類に適用し、知見を広げていくことが将来のプロセス開発に必要である。本論文は、粒径、炭化水素の抽出率、細胞強度、藻類の表面構造などを定量的に評価することで、*B. braunii* に最適な機械的破砕プロセスについて検討したものである。

論文は全 5 章構成であり、査読付き論文 3 報、審査中の論文 2 報分を含む、計 5 報分の内容である。

第 1 章は緒論であり、本研究の背景、既往の研究および目的を述べている。

第 2 章では、3 種類の破砕装置を用いて藻類を破砕し、炭化水素の抽出に対する細胞破砕の有効性を評価するため、3 種類の細胞破砕装置(高圧ホモジナイザー、ビーズミルおよびジェットペースタ)を用いて *B. braunii* の培養液を破砕し、炭化水素を抽出している。その結果、高圧ホモジナイザーでは細胞が大きく破砕されたのに対し、ビーズミルやジェットペースタでは破砕の程度が小さいか、または全く細胞が破砕されなかったことを明らかにしている。一方で、炭化水素の抽出率をみると、いずれも未処理の場合と比較して大きく増加したことがわかる。このことから、細胞を破砕するかどうかにかかわらず、コロニーを破砕するのみで炭化水素の抽出率が増加したことが示唆している。また、コロニー周囲の多糖類(EPS)をコロニーから除去することによっても炭化水素の抽出率が向上したことを明らかにしている。前章までの結果により、コロニーの破砕およびコロニー表面からの EPS の除去が湿潤状態の *B. braunii* からの炭化水素の抽出に有効であることを受けて、粒径分布およびゼータ電位を用いてコロニーの破砕度合いと表面構造の変化を同時に定量し、炭化水素の抽出率との関係を調査している。これらの実験から、EPS の剥離度合いをゼータ電位で定量化できることを示し、粒径分布を用いることでコロニーの破砕度合いを定量的に表現できることを示している。

第 3 章では炭化水素の抽出率に影響を及ぼす因子を検討し、炭化水素抽出率の予測方法について検討している。

第 4 章では、細胞個々の機械的強度を測定し、他の種の藻類と強度を比較した。細胞の機械的強度を理解することで、投入するエネルギーが過剰となることを防ぐことができる。そこでナノインデンテーション法を用いて細胞の機械的強度を測定している。アクリル基板に固定させた細胞を培養液中で垂直に加圧させ、細胞が破裂するまでの荷重と変位を測定している。この実験により、*B. braunii* の細胞強度はおよそ 60 MPa である一方で、細胞 1 g を破砕するために必要なエネルギーは *B. braunii* と比較して細胞強度が低い藻類よりも小さいことを明らかにしている。これは *B. braunii* が既往の研究で用いられている藻類よりも大きな粒径を有し、藻類 1 g を構成する細胞数が少ないためである。実用的には、細胞 1 g あたりの強度のほうが重要であるため、*B. braunii* は既往の研究の藻類よりも小さなエネルギーで破砕可能であることが示唆されている。

第 5 章は総括であり、各章の成果をまとめている。

以上の検討から、炭化水素の抽出率の促進に関わる *B. braunii* の機械的な破砕現象に対する実験的アプローチの発展に貢献し、微細藻類に対する細胞破砕の工学的なプロセス解析を推進した。

よって、本論文は博士(工学)の学位論文として合格と認める。